Lung Inflammation: Role of Endogenous Chemotactic Factors in Attracting Polymorphonuclear Granulocytes[1-4]

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SUMMARY The inflammatory reaction in the lungs can be considered a mechanism of host defense that augments local alveolar cellular and humoral defense against microorganisms and particulates which challenge the airways. As part of this reaction, the accumulation of blood inflammatory and phagocytic cells, primarily PMNs, and fluid components from plasma may be under control of chemoattractant factors and vasoactive mediators. From the air-side, chemotactic factors originating from alveolar macrophages or through activation of the complement system seem essential in initiating the influx of PMNs into the alveolar space. The kinetics of synthesis and release of chemotactic factors from alveolar macrophages of animals and humans and the status of their immunochemical analysis is the essence of this report. Coupling phagocytosis (afferent function) with its capacity to secrete several kinds of effector molecules (chemotactic factors, complement components, leukotrienes, and platelet activating factor), the alveolar macrophage is considered to have a pivotal role in overall regulation of the inflammatory reaction.

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Introduction

The airways of the respiratory tract are constantly subjected to foreign debris alighting on their surface, which is inhaled with ambient air or admixed with nasooropharyngeal secretions that are aspirated. However, the respiratory tract has an elaborate array of cleansing mechanisms that effectively remove this debris and, under usual circumstances, no harm occurs. These mechanisms are part of a broad umbrella of pulmonary host defenses that protect the health and integrity of the air-exchange surface of the lungs (1).

Counterattacking, therefore, the influx of airborne particles and microbes that enter the conducting airways are formidable anatomic barriers, such as the larynx and the branching structure of the airways, a perpetual ciliary clearance apparatus, locally secreted proteinacious substances containing mucus and immunoglobulins that coat the ciliated epithelium, and a cough mechanism. Further down in the terminal airways and alveoli, phagocytic cells, principally alveolar macrophages, scavenge debris reaching the actual air exchange area.

In sum, these surveillance mechanisms (2) efficiently guard and cleanse the respiratory tract. In addition, other augmenting mechanisms are available and may be operant only at crucial times to aid the usually effective surveillance apparatus. The capacity to make specific antibody in response to a micro-organism that at a future encounter would accelerate its clearance, or prevent attachment to the airway epithelium, is an example. The capacity to generate an appropriate inflammatory response in lung parenchyma is another (3). Thus the mobilization of inflammatory cells and fluid factors from the intravascular compartment into the lung is crucial for effective pulmonary host defenses.

Obviously, full mobilization of the inflammatory reaction leading to pneumonitis and clinical illness occurs infrequently in healthy individuals who are not exposed in an extraordinary way to massive inocula or unusually virulent microbes, but some lesser gradation of the reaction may be needed frequently to combat inhaled and aspirated particulates and microbes that incessantly plumb the depths of the airways. On the other hand, inappropriate or excessive inflammation in the lungs may create pathology that is part of many chronic diseases that affect the lungs. Certain allergic, vasculitic, and autoimmune diseases feature abnormal recruitment of inflammatory cells to the lungs as do a variety of toxic environmental exposures such as cigarette smoke, inhaled asbestos, and silica particles. Accompanying the cellular component of inflammation are its by-products in the form of lysosomal enzymes, oxygen radicals, and a myriad of proteases that can cause destructive injury. Important in the initiation and perpetuation of the inflammatory reaction is the role of chemoattractant factors that help direct and recruit inflammatory cells into extravascular spaces (4). The origin of such factors is multiple and they can arise from complement cascade activation, be synthetic products of cells, or be generated from the debris of inflammation itself that contains a mixture of senescent, injured leaking cells, and plasma protein components.

This report will concentrate on the chemotactic factors that can arise or be generated in the airways and alveoli, and that seem to have relevance in initiating an inflammatory reaction in response to airway challenge. The resulting inflammation is a controlled biologic reaction, we feel (3), and at times it may be a modest, self-limited reaction, perhaps undetectable clinically, but at other times it becomes significantly amplified so that clinical illness is perceived. When a specific microbial organism is the cause, pneumonitis appropriately describes the reaction; when the cause is a noninfectious one, or perhaps is of unknown etiology as is the case for many of the chronic alveolar-interstitial inflammatory diseases, alveolitis is a reasonable term. Where possible, we will emphasize the human situation but recognize the fact that experimental animal studies have been fundamental in dissecting and manipulating the inflammatory response and will remain indispensable for future work.

One final qualifying statement should be made as we embark on a review that will emphasize the role of polymorphonuclear neutrophils (PMNs) in the inflammatory response. We shall ignore the granulomatous-type reaction that prominently includes lymphocytes and transformation of macrophages into epitheloid cells, etc. Some lung research literature indicates that alveolar macrophages liberate factors that influence the accumulation of lymphocytes and that lymphocyte-derived factors affect macrophage motility. Certainly these mechanisms must be considered as well in developing a comprehensive view of the complex cellular mechanisms of lung inflammation.

Baseline—What Inflammatory Cells Are Already in the Airways?

Lung washing techniques that retrieve respiratory cells from the alveolar and distal airway surfaces of animals and humans are the only available means to sample normal lungs in a relatively noninvasive way. Analysis of cells so obtained, now reported by

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many investigators, reveals that the preponderance of cells are alveolar macrophages (<90%), perhaps 4 to 8% are lymphocytes, and 1 to 2% are PMNs. Rarely are eosinophils or basophils detected. These cells, of course, represent the detachable population of cells that are dislodged with infusion and aspiration of saline fluid introduced into the airways. This lavage by no means retrieves all cells present in the lung area sampled, and just how thorough or representative the washing sample is has not been correlated with actual histology in normal humans.

Recent data quantifying PMNs in the lungs of normal rabbits are relevant (5). PMNs constituted about 10% of the cells observed to be free in the alveoli, but only 2.5% of respiratory cells recovered by lung lavage were actually PMNs. It was estimated that lavage effectively removed about 42% of PMNs located in the air space (range of sampling efficiency was 28 to 56%). PMN counts in right and left ventricular blood samples were almost identical so significant trapping of PMNs in the vasculature was not believed to occur. About 6 × 10^4 air space neutrophils per g of lung tissue were calculated. Alveolar macrophages were not observed to phagocytose or to otherwise remove PMNs. Aged or senescent PMNs, however, can be ingested under *in vitro* conditions by macrophages, and this may still be a pathway for cell removal when studied more thoroughly.

To establish basal conditions, it is unclear how many PMNs are naturally present in vascular and capillary areas of normal nonsmokers' lungs as part of the marginated pool of PMNs, or how many are located in interstitial portions of lung parenchyma. Because the lungs contain a large capillary network that serves as an important filter at the interface of the arterial and venous compartments of the circulation, the availability of PMNs within the lung vasculature would seem to be an important feature of host defense. The PMN being an endstage cell with a limited lifespan of a few hours, the mere presence of tissue PMNs in the lungs suggests an active influx and turnover of these cells. In contrast, no other inflammatory cell type seems to be present in normal lungs in a sufficiently high number to make it an important candidate for inclusion in the inflammatory reservoir of the lung. Specifically, eosinophils come to mind. Clearly, eosinophils are important components of allergic lung diseases and various eosinophilic syndromes, but they are not a prominent part of the usual bacterial infection–pneumonitis response. Moreover, as we shall see, the specific chemotactic factors liberated by alveolar macrophages have consistently been weak and negligible stimuli for eosinophils.

Few PMNs have been found in human lung lavage specimens; this paucity was noted in the early studies that used normal subjects (6–9). As illustrated with our group of nonsmoking volunteer subjects (table 1), the differential cell count of respiratory cells recovered with BAL shows that 1.5% of the cells were PMNs (10). For the smoker groups, the percentage increased to 2.4 and 3.7. These increases become more impressive when they are related to the total cell yield and are presented as the total PMN count (figure 1). All nonsmokers had less than 10^6 PMNs per lavage cell total, and 10 of 34 had no evidence of PMNs among their lavage cells. Most marijuana and tobacco smokers had a logarithm greater PMN count than nonsmokers. Values in males and females did not differ. Thus, smokers in general would seem to have a heavier burden of PMNs in their airways, but there are exceptions. About 13% (7/56) of smokers had no PMNs among the cell mixture and another 16% (9/56) had counts similar to those of nonsmokers. As alluded to, the lavage procedure may wash out only part of the PMN population and more remain behind in the alveoli.

TABLE 1
DEMOGRAPHIC AND LUNG CELL RESULTS IN NONSMOKERS AND SMOKERS

Groups	Smoking: Age (yr) Sex	Smoking: HX Pk-Yr	Smoking: Cell Count (× 10_4)	Differential (%): AM	Differential (%): PMN	Differential (%): Lymphs	Differential (%): EOBASO*
Nonsmokers (n = 44)	27.2 ± 1.2† 27M 17F	0	19.8 ± 2.3 (3–59)‡	87.4 ± 1.3 (77–99)	1.5 ± 0.2 (0–5)	9.3 ± 0.8 (0–23)	—
Marijuana Smokers (n = 7)	20.3 ± 0.7 6M 1F	—	63.4 ± 10.0 (27–99)	88.5 ± 2.6 (76–100)	3.7 ± 0.9 (0–10)	7.7 ± 2.2 (0–20)	—
Light Smokers (< 10 pk-yrs) (n = 18)	23.4 ± 0.7 11M 7F	6.4 ± 0.6	58.2 ± 5.8 (27–110)	93 ± 0.9 (79–99)	3.3 ± 0.9 (0–17)	4.0 ± 0.5 (0–10)	—
Moderate Smokers 10 to 20 pk-yrs (n = 14)	25.8 ± 0.9 7M 7F	15.8 ± 0.9	91.1 ± 16.3 (27–234)	95.7 ± 0.7 (85–99)	2.4 ± 0.4 (1–9)	2.5 ± 0.4 (0–6)	—
Heavy Smokers (> 20 pk-yrs) (n = 20)	38.2 ± 2.3 12M 8F	41.1 ± 5.9	102.0 ± 12.1 (15–200)	92 ± 0.5 (75–99)	3.5 ± 0.6 (1–18)	3.7 ± 0.5 (1–10)	—

* Less than 1% in all differential counts (from reference 10).
† Values are mean ± SEM.
‡ Range observed.

We decided, therefore, to see if there was any substance in the BAL fluid that might stimulate or attract inflammatory cells and could account for their influx into alveoli (12). The progressive increase of inflammatory cells and the relative potency of concentrated BAL to attract these cells *in vitro* are illustrated (figure 2) from a monkey serially lavaged during a 72-h interval. Each lavage was done through a fiberoptic bronchoscope placed in the same portion of lung. In the third lavage fluid,

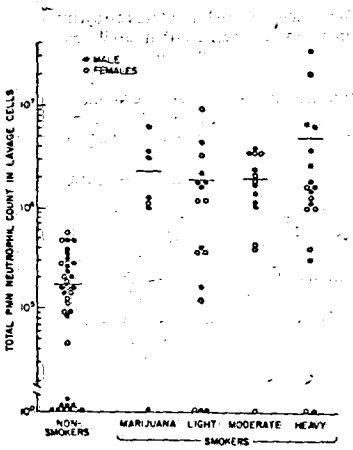

Fig. 1. Total numbers of PMNs in the respiratory cell pellets obtained by BAL are given for nonsmokers and groups of smokers. Smokers are divided according to marijuana use (no tobacco) and to history of tobacco inhalation exposure: light have < 10 pk-yr use, moderate have between 10 to 20 pk-yr use, and heavy have > 20 pk-yr use. Horizontal bars denote the mean value (from reference 10).

Identification of Chemotactic Substances in Airways of Animals

As an incidental finding, an awareness that inflammatory cells accumulated in ostensibly noninfected airways and alveoli developed as we were trying to characterize respiratory lymphocytes in bronchoalveolar lavage (BAL) fluid from monkeys (11). In the course of relavaging monkeys at daily intervals to procure additional lymphocytes for T- and B-cell identification, we noted a striking increase in the percentage of PMNs in the lavage fluid. Yet there was no obvious infection or irritation in the airways of the animals, which seemed to be healthy and without evidence of pneumonitis by chest radiography or physical examination.

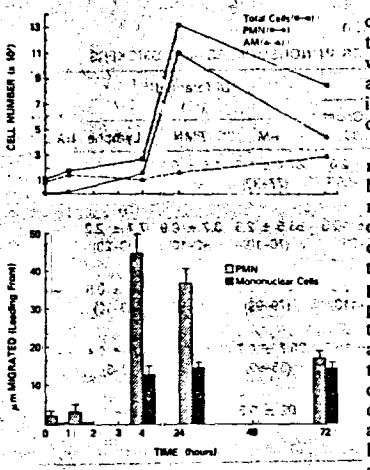

Fig. 2. A monkey underwent BAL at intervals during a 72-h period to illustrate changes in the total number of recoverable respiratory cells and in the number of PMNs and alveolar macrophages (AM) (*upper panel*). Simultaneous chemotactic activity, expressed as migration of the leading front of cells into a micropore filter, in concentrated lavage fluid was measured for 2 cell preparations (*vertical bars*) (from reference 12).

obtained 4 h after the initial one, the total respiratory count had doubled and PMNs accounted for about 45% of the cell population; when sampled at 24 h, a tenfold increase had occurred in recoverable cells of which 90% were PMNs.

When cell-free BAL fluid was assayed for chemoattractant activity that would cause directed motion of PMNs and purified monocytes into a micropore filter, considerable activity was found in the 4- and 24-h specimens. Activity in the BAL specimens was equivalent to that generated by lipopolysaccharide-activated serum and caseinate that were known to be potent chemoattractant substances. To isolate and characterize the active substance(s) in BAL, a pool of lavage fluid obtained from 5 monkeys lavaged for the fourth time after the initial lavage was chromatographed over a calibrated Sephadex G-75 column, and chemoattractant activity in the effluent fractions from the column was assessed for PMNs and mononuclear cells (figure 3). Chemotactic activity was detected in 2 areas of the elution profile: Peaks A and C, which coincided with an elution position of a 15,000-dalton marker, and Peak B, which approximated a 5,000-dalton marker. Peak B activity seemed selective for PMNs. The active fractions were pooled and characterized further. For pool A, heating (56° C for 45 min) did not diminish chemotactic activity, whereas the addition of an anti-C_3 antiserum decreased the relative activity by 45%. Such information led to the conclusion that chemotactic activity found in these chromatographic fractions of BAL was due to a complement fragment, probably C_3a. Left unaccounted for was activity in Pool B, which was unaffected by anti-C_3 or C_3 antiserum but was heat labile.

We considered that Pool B's activity might arise from a cellular source. Because lavage of normal lungs yields predominantly alveolar macrophages (>90%) and only a few lymphocytes and PMNs, *in vitro* culture of macrophages was the first choice to study. When glass-adherent macrophages were additionally activated with a phagocytic stimulus of opsonized bacteria, the culture supernatant possessed considerable chemotactic potency. Intermediate potency was obtained from adherent, but otherwise unstimulated cell cultures, but decidedly less activity was generated from alveolar macrophages that were tumbled to keep them in suspension and prevent cell adherence.

Thus macrophages seemed to produce a chemoattractant substance spontaneously when cells were allowed to adhere in cultures; however, a phagocytic stimulus quantitatively enhanced the amount. Moreover, incubation of a macrophage monolayer with cycloheximide, which putatively diminished protein synthesis by cells, largely inhibited the formation of chemotactic activity in the culture medium of bacteria-stimulated cells. Hypotonic lysis of a pellet of macrophages, however, revealed chemotactic activity in the cell lysate, indicating some storage of the factor within the cell. Chemotactic substance for alveolar macrophages was concluded to be in part preformed and stored in the cells and hence available for immediate release; however, additional factor apparently could be synthesized, providing a mechanism for sustained release.

To characterize this macrophage-derived chemotactic substance, culture media was collected after 8 h of cell stimulation, an interval that yielded the most chemotactic activity in cell supernatants, concentrated and gel-filtered through the Sephadex G-75 column as shown in figure 3. Effluent fractions with chemotactic activity, again assessed in terms of migration of the leading front of cells into a micropore filter, centered around the 400 to 420 elution volume, precisely coinciding with Peak B. Activity was selective for PMNs and ineffective for mononuclear cells or eosinophils. Heating the pooled effluent fractions diminished chemotactic activity, but anti-complement antiserum did not. This factor seemed realistically to be a product of adherent macrophages in culture and not from another cell type. The adherent cell cultures were virtually all composed of alveolar macrophages (>98%), although a few adherent lymphocytes could not be rigidly excluded; other inflammatory cells such as PMNs were absent. At the time, it was uncertain if this

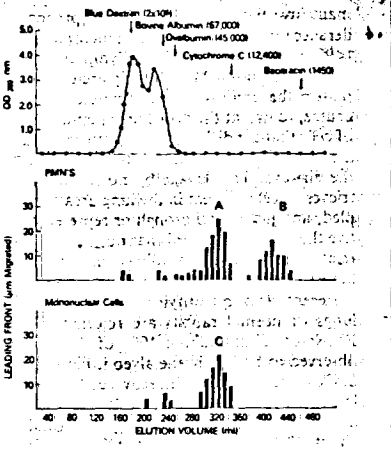

Fig. 3. The elution profile of concentrated BAL fluid from a Sephadex G-75 column is shown. Protein is located in the upper panel (OD280nm) and the elution positions of proteins with known molecular weights are given. Chemotactic activity in the column fractions, depicted on the ordinate as micrometers of migration of the leading cell front into a micropore filter, is shown for PMNs (*middle panel*) and for mononuclear cells (*lower panel*). Concentrated BAL fluid has two peaks of chemotactic activity for PMNs that eluted at 320 ml (Peak A) and at 410 ml (Peak B), and one peak of chemotactic activity for mononuclear cells that also eluted at 320 ml (Peak C) (from reference 12).

small molecular weight factor released from *in vitro* cultured alveolar macrophages was unique or possibly related to other cell-associated chemotactic factors such as dialyzable transfer factor (13) or an eosinophilotactic tetrapeptide (ECFA) (14). However, the macrophage factor consistently showed no appreciable activity for eosinophils. Moreover, the small size of the factor seemed to differentiate it from other substances with biologic activity known to be secreted by macrophage (15).

Within the same laboratory, namely the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD, other colleagues also were looking for endogenous chemotactic substances that could produce an inflammatory response in lung tissue. Hunninghake and associates (16), working with an inbred strain 13-guinea pig system, identified a chemotactic factor generated by alveolar macrophages after phagocytosis of heat-killed *Staphylococcus aureus*. Characterization of this factor in lung lavage fluid found it to elute from a calibrated Sephadex G-75 column in the position of <5,000-dalton marker and to be resistant to heating at 56° C for 45 min, but totally destroyed after incubation at 100° C for 10 min. For *in vitro* cultured alveolar macrophages, a bacterial stimulus enhanced factor secretion, although an appreciable amount was

produced spontaneously by the adherent cells; inhibiting protein synthesis by the cells likewise diminished the amount of chemotactic activity generated in the culture supernatant by postphagocytic macrophages. This study extended prior observations in the monkey model discussed (12) in 2 important ways. First, it demonstrated that an intratracheal injection of heat-killed *S. aureus* (10^{10} lung dose/pig) produced a significant *in vivo* PMN inflammatory response. Second, when the active factor, generated *in vitro* from macrophages, was instilled intratracheally, a significant increase in PMNs in lung BAL was found, suggesting an *in situ* role for this chemotactic substance.

Compared with the saline-injected control guinea pigs that developed about 0.5×10^6 PMNs in lung lavage, the animals that received macrophage-derived chemotactic factor had about 2×10^6 PMNs per lavage. Consistently, this factor, like the monkey macrophage factor, preferentially attracted PMNs and had no effect on eosinophils and other inflammatory cells. From photomicrographs of the lungs, obvious alveolar hemorrhage was not produced nor elicited by the chemotactic specimens. This latter finding has not been stressed enough, I believe, because the lack of appreciable erythrocyte leakage into the alveolar spaces distinguishes this mechanism of lung inflammation from that generated by the airway instillation of preformed immune complexes (17, 18) or of C_5a (19), both of which cause alveolar hemorrhage and suggest a more fulminant and possibly injurious lesion. The macrophage-induced chemotactic stimulus seemed to produce a more discreet and specific PMN accumulation in alveolar spaces.

Using the same guinea pig model, Gadek and colleagues (20) delved more thoroughly into the kinds of stimuli that induce alveolar macrophages to release the PMN specific chemotactic factor. A variety of particulates was used to stimulate adherent macrophages under tissue culture conditions that included heat-killed *S. albus*, zymosan particles, Sepharose beads, and IgG containing immune complexes. As noted in previous studies, physical adherence of macrophages to a surface was a sufficient stimulus for chemotactic factor production, but the kinetics of its formation were slow and did not reach a maximum until 18 h in culture had elapsed. In contrast, this activity after particulate stimuli developed much faster and was quite evident in the 3-h supernatant specimens.

The greatest chemotactic activity was produced by the immune complexes and IgG coated erythrocytes; intermediate activity was found with the microbial stimuli. Furthermore, if the zymosan particles were preincubated with normal serum and then fed to macrophage cultures, the output of chemotactic factor was twofold greater. The active component in serum was found to be C_3b, which complexed to the particulate antigen and probably enhanced attachment to the surface of the macrophage. Evidence suggests that C_3b was produced by the alternate complement pathway that is most likely the dominant pathway operating in the normal airway (3). These findings that IgG and C_3b containing stimuli elicit maximal output are not surprising, knowing that alveolar macrophages have special receptors for IgG Fc and C_3 (21). Likewise, specific receptor binding would ensure good particle attachment to cell membranes and facilitate phagocytosis. Chromatography on Biogel P-2 of macrophage cell culture supernatant separated chemotactic activity into 2 elution positions corresponding to molecular weights of 400 to 800 daltons.

Evident in all the monkey and guinea pig alveolar macrophage experiments was the high background of spontaneous secretion of chemotactic factor into culture medium. In part, cellular attachment to the culture chamber is responsible for activating the macrophages and increasing baseline metabolic function; preventing cell adherence by tumbling them in culture greatly diminishes the production of chemotactic activity. Although ingested particles, bacteria, and immune complexes can appreciably enhance chemotactic factor secretion, it is evident that secretion of the factor is largely nonspecific. Moreover, once liberated, this chemoattractant activity generated by macrophages may be generalized rather than local and selective. Some recent data support this view.

In our initial monkey lavage experiments (12), the same portion of the lungs was repeatedly lavaged through a main stem bronchus; other nonlavaged lobes were not systematically sampled for influx of inflammatory cells. Likewise, guinea pigs were injected intratracheally with a milliliter of fluid containing macrophage-derived chemotactic factor, rather than selective placement of the sample, and then whole lungs were lavaged to recover cells. Cohen and Batra (22), using dogs, found that repeated washing of 1 lung segment initiated an influx of inflammatory cells into unwashed segments, even in the contralateral lung. Thus, unilateral lung lavage produced a bilateral PMN response, always greater in the ipsilateral area washed but appreciable in the contralateral segment too. In fact, repeated placement of the wedged bronchoscope (5 mm size) in a sublobar bronchus produced a PMN rich influx into the area, demonstrating that actual lavage was not a necessary requirement for attracting PMNs to the alveolar surface and that a foreign body or irritant, in this instance the bronchoscope, would suffice. Minimal influx of PMNs also was noted in a couple of dogs (2/8) when the contralateral lung that had not been invaded with the bronchoscope was lavaged and cells analyzed. The experiments showed that local sublobar lung lavage had widespread alveolar effects in that repeated lavage or just placement of the bronchoscope could evoke a PMN inflammatory response in a specific lung area and in distant, removed ones as well, indicating that a general stimulus must be liberated, possibly a neurogenic or reflex one. The effect was not limited to lung parenchyma because blood leukocytosis and bone marrow release of young forms of PMNs also occurred, no doubt to supply an adequate number of PMNs for egress into lung. More than just a macrophage chemotactic factor was operant in this large-scale response. These investigators also found that monkeys responded in a similar way. Moreover, they selectively instilled concentrated lung lavage fluid into the right or left bronchus intermedius of monkeys and found that this fluid elicited a transient but 240% increase in peripheral blood PMNs, confirming a systemic effect on the bone marrow reserves from the lung focus. However, an identification of active factors present in the lavage fluid was not attempted.

Human Alveolar Macrophage Factors

As expected, the search for inflammatory mediators in the airways quickly moved to the human lung and focused on the alveolar macrophage. Lung lavage of normal volunteers produced a high yield of macrophages (> 80% of respiratory cells recovered) from cigarette smokers and nonsmokers (10). Because the monkey and guinea pig studies had identified a macrophage-derived chemotactic factor, it was reasonable to determine whether human cells made the same. Merrill and colleagues (23) found this to be the case. *In vitro* cultured alveolar macrophages produced chemoattractant activity in cell free supernatant after surface attachment. But peak activity did not occur until 22 h in culture if the cells were not stimulated further. Preventing attachment by tumbling the macrophages greatly retarded the production of chemotactic activity, but after 22 h comparable amounts of activity were present. However, when adherent macrophages were stimulated with an IgG-containing immune complex that attached to their cell surface, chemotactic activity was detected in the first supernatant sample at 3 h and in maximal amount. The quick burst of activity tapered off and was decidedly less potent in the 22 h sample. Zymosan-particles that gave a microbial phagocytic stimulus produced intermediate amounts of chemotactic activity, reaching maximal activity by 6 h, but in contrast sustained the degree of activity into the 22-h supernatant specimen. Thus the kinetics of chemotactic activity produced by the macrophage-predominant cell cultures (>95%) showed that cell adherence alone was a sufficient stimulus to cause activity and preventing adherence retarded it significantly, but did not prevent it entirely.

Of course, mechanical agitation of the cells to prevent surface attachment should be considered a form of stimulation and is not a perfect control situation, especially when compared with the macrophages' in situ position on the alveolar surface. Lung movement obviously occurs with ventilation, but the rhythmic, less violent expansion-contraction of the alveolar surface occurring with tidal breathing and modulated by surfactant and other secretions may be far different from a rotating, plastic tube and sloshing medium. Of interest, approximately 2 wk in longterm culture was required before alveolar macrophages seemed to calm down and produce no detectable chemotactic activity in their culture medium. Poor nutrition and marginally viable cells were not the entire explanation, because cellular stimulation with IgG complexes produced maximal secretion of chemotactic activity from the 2-wk-old macrophage cultures. Like macrophages from other species, the chemoattractant substance(s) was relatively specific for inducing PMN motility and decidedly less potent for mononuclear cells.

To characterize the chemotactic activity produced by monolayers of alveolar macrophages from noncigarette smokers, supernatant specimens from unstimulated cell cultures were collected, concentrated, and chromatographed on a calibrated Sephadex column (figure 4). Eluent fractions containing chemotactic activity for PMNs centered about the elution position of a 9,500-dalton material and an approximately 1,000-dalton marker. In similar studies using supernatant from zymosan-stimulated macrophages, the same 2 peaks of chemotactic activity were detected in the elution profile, but the relative potency of the activity was somewhat different, shifting to more of the smaller molecular factor. This suggested that acute cell activation might change the relative proportions of factor secretion or indicate that the smaller substance was a breakdown product of the larger.

The relationship between these 2 possible chemotactic factors was not elucidated completely. Further analysis of the larger molecular weight factor indicated it was susceptible to proteolytic degradation with trypsin that diminished its activity. Isoelectric focusing in polyacrylamide gels of an ^{125}I trace-labeled specimen actually revealed the "homogenous" factor to disperse into a multiple band profile, but with chemoattractant activity confined to a single peak with a pI of 5.0 (peak B). Various inhibitors, including anti-C_5 and -C_3 antisera, confirmed the integrity of this 9,500-dalton chemotactic factor to be non-complement in nature. But blocking prostaglandin metabolism in the cells did inhibit early release of chemotactic activity by macrophages, and it was considered probable that some activity attributed to the small molecular weight substance could represent a lipoxygenase pathway product.

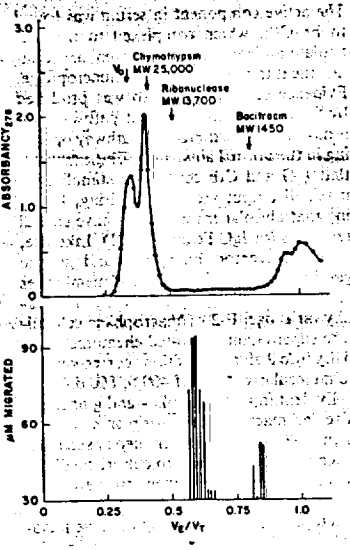

Fig. 4. Gel filtration of pooled AM culture supernates. One hundred fifty ml of unstimulated AM culture supernate from nonsmokers were concentrated to 7 ml and filtered through Sephadex G-50 SF (column dimensions 2.6 (i.d.) × 61.4 cm with gel bed volume of 324.6 ml) in phosphate-buffered saline, pH 7.2. The position of eluent fractions (4.5-ml fractions were collected) is expressed as V_E/V_T. In Panel A, the relative protein content (A_{278}) of the fractions and the elution position of calibration markers are given. In B, migration of PMNs in micrometers toward these fractions is shown. Control cell migration in the buffer was 30 μm. The column apparatus was sterile and eluent fractions were free of detectable endotoxin material (from reference 23).

Concomitantly, Hunninghake and colleagues (24) were analyzing their human alveolar macrophage-derived chemotactic factor. The results were complementary but slightly different, although the basic methodology used was generally similar. BAL from healthy nonsmokers recovered respiratory cells, principally alveolar macrophages (mean 92%), that were cultured and stimulated with a variety of particles, opsonized particulates, and immune complexes (bovine serum albumin–IgG anti-BSA antibody). Active cell supernatants were chromatographed through Sephadex G-25 or Biogel P-2 gel media in phosphate-buffered saline. Cell supernatants were generally harvested 3 h after specific stimulation for assay. Just as we noted (23), special macrophage stimulation could enhance the release or secretion of chemotactic activity in several hours; however, with extended culture (18 h), nonstimulated control cells eventually produced maximal activity. All particles stimulated chemotactic activity versus controls, but opsonization of the particles provided an additional increment, especially with heat-killed *Staphylococcus aureus*. IgG-containing complexes were particularly potent, but complement receptor attachment to the macrophages' surface was not an efficacious stimulant.

After fractionation of active cell culture supernatants, chemotactic activity was consistently found in the 400 to 600 dalton elution range of the calibrated columns. Detailed characterization of this material found it to: be stable on heating at 56° C and 100° C, exhibit stability over an enormous pH range (pH 1.0 to 12.0), resist a variety of proteolytic enzymes, contain 2 major isoelectric points (PI 7.6 and 5.2), and be extracted by organic solvents, suggesting it contained some lipid components. In terms of functional activity, the material was preferentially active for directing migration of PMNs compared with that of monocytes and eosinophils. Moreover, macrophage supernatants containing chemotactic activity induced normal human PMNs to release lysozyme and lactoferrin.

In summary, several small molecular size chemotactic factors have been conclusively demonstrated and characterized. These factors are compared (table 2) for the various species of alveolar macrophage studied. It seems that human alveolar macrophages can secrete at least 2 well-defined chemotactic factors that show selective activity for PMNs. The small < 1,000-dalton factor is in part a lipid-containing substance and may represent a lipoxygenase pathway metabolite of arachidonic acid. This possibility is relevant because one such substance, 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid, has been found to stimulate random and directed migration of PMNs and eosinophils (25, 26).

To give credence to the concept that an alveolar macrophage-derived chemotactic factor might participate in the inflammatory reaction in human lung diseases, Hunninghake and colleagues (27) sought evidence of chemotaxin secretion by alveolar cells lavaged from patients with idiopathic pulmonary fibrosis (IPF). This was a relevant disease to study because a low-grade alveolar inflammation is often present, especially in an early, cellular stage of disease. In the initial studies analyzing bronchoalveolar lavage fluid from IPF patients (28, 29), it was noted that among the respiratory cells retrieved from untreated patients, PMNs accounted for a consistent percentage (mean about 30%, 5 to 80 range observed) of the cells. A small percentage of eosinophils (about 4%) also was characteristic. Moreover, PMNs correlated with cellular reactivity in biopsy specimens (28) and with gallium lung scanning that generally reflects isotope uptake by inflammatory cells and activated macrophages (30).

A group of 15 patients with IPF, considered to be in midstage of their disease, were investigated for possible secretion of

TABLE 2
COMPARISON OF ALVEOLAR MACROPHAGE DERIVED FACTORS

Origin	Size	Stability	Characteristics
Monkey[12]	5,000 daltons	Heat labile	Attracted PMNs not monocytes or eosinophils
Guinea pig[16]	1,500 daltons (600 d)	Heat stable	Generated after *in vivo* phagocytosis, had *in vivo* chemotactic activity
Human[23,24,27]	8,500 daltons	Sensitive to trypsin and heat stable	PMNs attracted PI 5.0
	< 1,000 daltons (600 d)	Resistant to trypsin and proteases, heat and pH stable	PMNs attracted in preference to monocytes and eosinophils; promoted release of elastase and lysozyme from PMNs
		Sensitive to serum chemotactic factor inhibitor	PI 7.6 and 5.2 in part lipid, but not HETE

alveolar macrophage-derived chemotactic factor and compared with 8 nonsmoker normal volunteers (27). After BAL to retrieve respiratory cells from both groups, the cells were submitted to Hypaque-Ficoll density centrifugation to remove PMNs; the purified mononuclear cells, principally alveolar macrophages, were cultured for 3 h after which supernatant fluids were harvested and evaluated for chemotactic activity. Various stimuli were given to the macrophage cultures in the form of concentrated BAL fluid from IPF patients, Sepharose 4B beads, and IgG coated ox erythrocytes. Results of respiratory differential cell counts in the patients showed that 9 of 15 had greater than 10% PMNs among the cells (approximately 35%), and that the other patients had less than 10%; the normal nonsmokers did not have PMNs. Once in culture, macrophages from IPF patients with > 10% PMNs in their original mixture of lavage cells spontaneously released chemotactic activity into the culture medium, whereas cells from those with fewer than 10% PMNs generally did not release activity, nor did the cells from nonsmokers.

This just may have reflected nonspecific activity by the macrophages, and an interesting control would have been secretion by other intrinsically active macrophages as obtained from sarcoidosis patients or age-matched smokers. Nonetheless, the IPF macrophages clearly generated large amounts of chemotactic activity quickly (by 3 h); when this activity was localized in the chromatogram (Sephadex G-25 gel) of the supernatant fluid, it was found in the 400 to 600 molecular weight range fractions eluted from the calibrated column. Further analysis of this material showed preferential chemoattractant activity for PMNs compared with blood monocytes, and this activity was largely extractable with organic solvents, indicating a significant lipid component to be present. Thus, Hunninghake and colleagues succeeded in identifying the same kind of small molecular weight, partially lipid-containing chemotactic factor they had previously found from normal alveolar macrophages (24).

These investigators looked further for a mechanism that could account for the secretion of chemotactic factor by IPF macrophages, an important cell component of the inflammatory, cellular milieu of active alveolitis. The stimulus seemed to be in the form of an immune complex based on the following evidence: (*1*) patients with serum titers of immune complexes generally had alveolar macrophages that released large amounts of chemotactic factor, (*2*) lavage fluid obtained from patients with immune complex titers in serum could stimulate normal macrophages to secrete the factor, (*3*) actively secreting macrophages had partial blockage of their surface IgG Fc receptors, suggesting concomitant binding of immune complexes, and (*4*) intracytoplasmic granular deposits of IgG, identified with immunofluorescent antiserum, were in the actively secreting macrophages. In BAL fluid for 9 of 10 IPF patients with actively secreting macrophages, C_{1q} binding was increased, compared with fluid from normal subjects and from IPF patients whose macrophages did not release chemotactic factor, indicating that IgG complexes were present. Enumeration of immunoglobulin secreting cells (B-lymphocytes) in BAL was not determined for these patients (31, 32), so the origin of the complexes, whether from peripheral blood or local secretion in lungs, was not known.

This study indicates that a chemotactic factor was released from alveolar macrophages of patients with IPF who were in an active phase of alveolitis with PMNs present in bronchoalveolar lavage fluid. IgG immune complexes appear to be an important macrophage stimulus. Thus an endogenous, airside chemotaxin can be released quickly within a few hours and this capability correlates with an elevated PMN count (> 10% respiratory cells). It is important that excessive chemotactic factor secretion has been found in a special disease in which some inflammatory cell reaction is a usual pathologic finding. The mechanism offered seems reasonable that chemotactic factor released from alveolar macrophages can attract PMNs to the lung. In contrast, a diminished, insufficient inflammatory response in the lungs is often noted in patients or animals that are immunosuppressed and have a propensity to develop bacterial respiratory infection. Often profound granulocytopenia and a depleted bone marrow supply of PMNs are the ready explanations. Impaired alveolar macrophages, specifically a diminished capacity to generate chemotactic factor activity, also could contribute to a depressed lung inflammatory response.

Pennington and Harris (33) examined that question with a guinea pig model in which cyclophosphamide (15 mg/kg weight/day) or cortisone acetate (100 mg/kg weight, subcutaneously) treatment was given for 7 days. Alveolar macrophages were then obtained by lung lavage and placed in tissue culture. Adherent but otherwise unstimulated cells were left in culture for 24 h and at this time (for no activity was found in 4-h cultures) cell-free supernatant was assayed for chemotactic activity for PMNs. Both treatment regimens given for a week caused approximately a 25% decrease in the potency of the macrophage supernatant to attract PMNs. It was concluded that chemotactic activity produced by macrophages could be adversely affected and this might blunt the inflammatory response. Because therapy with corticosteroids does favorably influence PMN counts and inflammation in the airways of patients with cellular forms of IPF (29, 32), suppression of endogenously produced alveolar macrophage chemotactic factor might result. For the IPF patients in the series of Hunninghake and coworkers (27), 3 patients in the high and the low intensity alveolitis groups were receiving corticosteroids when studied, but the macrophage output of chemotactic substance was not compared in these respective specimens. Thus the Pennington and Harris (33) observation has not been extended to a human situation in which corticosteroid therapy was given.

Other Chemotaxins, Potentially of Alveolar Macrophage Origin

A large array of biologic substances has been attributed to synthesis and secretion by macrophages (15, 34, 35). In a recent count, over 50 things had been identified (34) and it is probable that new entries will be added. However, only 2 substances in the current list seem to have clear chemotactic activity that would qualify them for serious consideration as other endogenous chemotaxins produced by alveolar macrophages. They are C_5a and certain leukotrienes derived from arachidonic acid metabolism.

The role of the complement system in host defense of the airways and alveolar surface remains controversial. Properdin

Factor B (36) and a number of other complement components have been identified in lung lavage fluids (9, 29, 37) which support the concept that alternate pathway activation may be important in the lung. We reviewed this topic in detail in Reference 3. Interest focuses primarily on C_5a and C_5a des arg, considered to be the most potent inflammatory mediators and chemotactic molecules liberated by the complement cascades. These are capable by themselves of causing acute airway hemorrhage and exudation after intratracheal instillation (18, 19, 39, 40). If direct activation of alternative complement pathway actually occurs in the airways, then it is reasonable to postulate that a variety of inhaled or aspirated particles and microbes that gain access to the respiratory tree can trigger the complement cascade; C_3 and C_5 plus other intermediate components like the trimolecular complex C_{567} could be liberated and produce a number of fragments with chemoattractant properties. Quantitatively, C_5 derivatives seem to be the most effective and potent of the complement group.

Instead of generating one of these phlogistic fragments from the complement cascades, the precursor component normally may be present in airway lining secretions. We certainly found C_5 activity in concentrated BAL fluid obtained with serial lavage of ostensibly normal monkeys (12). Kolb and coworkers (41) also found antigenic and functional C_5 activity in concentrated BAL fluid from cigarette smoke-exposed and normal nonhuman primates (baboons). C_5 hemolytic activity in BAL fluids actually was almost twofold higher than in corresponding serum samples (C5H50 titers per albumin concentration were about 800 in BAL in contrast to mean values of 450 in serum), and resistant to inactivation by exogenous trypsin. These results indicated that the intact C_5 precursor molecule is present in the alveolar compartment. In contrast, preliminary studies looking for C_5 in human lavage fluids from normal nonsmokers (42) have noted a paucity of this factor, compared with serum, raising the probability that appreciable diffusion of C_5 from the vascular space into the lower respiratory tract must occur before a sufficient amount is present for activation.

Another possibility is that complement components are directly added to respiratory secretions from local synthesis in the lung; some evidence supports a cellular source for this. Scherzer and colleagues (43) have found that *in vitro* cultured human alveolar macrophages synthesize and secrete into cell supernatant fluids, functional C_3 and C_5, as assessed by a hemolytic assay and further identified antigenically. Pennington and coworkers (44) also have assessed the production of complement factors by *in vitro* cultured human alveolar macrophages; specifically, they looked for C_2, C_3, C_4, C_5, and factor B. These macrophages were lavaged from patients with a variety of diseases including sarcoidosis, squamous cell carcinoma, and recurrent pulmonary infections. Most cell specimens produced functional C_2 and factor B, and several made C_3; none produced C_4 and C_5. It seems evident that cultured alveolar macrophages can spontaneously produce a variety of functionally active complement components that are linked with the classical and with the alternate pathways. It is plausible to believe that the cells can add these components directly to the fluid milieu of the alveolar surface and hence be available to interact with deposited, inhaled debris and micro-organisms. Questions still left unanswered are the quantitative nature of the secretion, whether it is regulated or spontaneous, and how specific the response can be when stimulated by phagocytosis or immune complexes attaching to the cell's surface. Moreover, C_5 does not seem to be the sole complement component produced and it may not be made in predictable amounts.

Considerable interest is focusing on other macrophage products that have potential phlogistic activity: platelet-activating factor and certain products of lipoxygenase pathway of arachidonic acid metabolism. Because this work is just emerging like the complement work, an exhaustive review of largely preliminary publications is premature. The leukotriene products, derived from oxidative metabolism of arachidonic acid along the lipoxygenase pathway, have spasmotic properties that can cause bronchospasm, and one of the principal mediators of allergic Type I asthma, a slow reacting substance of anaphylaxis (SRSa), has been found to contain 3 leukotrienes: LTC_4, LTD_4, and LTE_4 (45, 46). Initial evidence suggests that alveolar macrophages from rats (47, 48) may secrete small amounts of SRSa and thereby be an alternate source of this mediator. On the basis of work with human PMNs, stimulation will produce several lipoxygenase products that have chemotactic properties for other inflammatory cells, namely 5-monohydroxyeicosatetraenoic acid (5-HETE), and 5,12-dihydroxyeicosatetraenoic acid (LTB_4). Such products have not been identified conclusively in alveolar macrophages; however, the low molecular weight chemotactic factor released from human alveolar macrophages, as characterized by Hunninghake and colleagues (24, 27), is partially lipid in nature and may constitute part of a HETE-type moiety. LTB_4 is, perhaps, of even more interest for it is a potent chemotactic factor with activity that is comparable with C_5 (49). Undoubtedly the potential role of these substances (50) must await their identification in appropriate cells that can initiate the inflammatory reaction in the airways.

Perspective: Role of Endogenous Chemotaxins

As already mentioned, the capability of generating an inflammatory reaction in the alveoli and on distal airway surfaces of the lung is a fundamental element of normal respiratory host defense. Yet full mobilization of the reaction is seldom needed; overt pneumonia, as an example, is an infrequent occurrence in the healthy person. Thus individual, well-controlled components of the overall defense system suffice in most instances to clear or contain foci of debris and infection.

Exclusion of foreign substances inhaled with inspired air is obviously very efficient, and the usual surveillance mechanisms operant in the nose, throat, and conducting airways are effective in intercepting these things. On the air exchange surface, alveolar macrophages are assumed to be the scavenger and phagocytic cell front line that cleanses this area, but this may not always be the case as the particular particle or microbe can be handled in different ways. Amply shown in aerosol-exposed animal models that feature reproducible deposition of bacteria in the lungs (51–53), certain bacteria, such as *Streptococcus sanguis* and *Staphylococcus aureus*, are contained by alveolar macrophages; whereas others require a combined macrophage and PMN response, as found for *Streptococcus salivaris*, or elicit a predominantly PMN response in the case of *Neisseria catarralis* or *Klebsiella pneumoniae* and *Escherichia coli*. In general terms (54), the gram negative organisms containing lipopolysaccharide cell wall components evoke more inflammatory response with PMNs than gram-positive ones, a distinction of some relevance in assessing which factors are important in initiating the host's inflammatory reaction (figure 5).

Once in the alveolus, a bacterium might stick to the lipid (surfactant) proteinacious lining secretions that are thinly spread over the alveolar surface and may soon be ingested by a macrophage. However, several possible things could happen. The bacterium, an encapsulated one of a gram-positive (GR+) or gram-negative (GR−) variety, could become coated with a mixture of immunoglobulin (? antibody), surfactant, and fibronectin, or interact directly with available complement pathway proteins. Proteases (and lipopolysaccharide from the gram-negative organism) from the bacterium could be liberated as a consequence and activate or degrade any parts of the mixture just mentioned. What ensues might not always be predictable. The complement sequence could be activated (alternate pathway) and C5a or C_{5a} desarg generated directly from a precursor component, or opsonic antibody and fibronectin could facilitate phagocytosis by the macrophages that would lead to bactericidal killing of the microbe. Once stimulated, the macrophage could enhance its secretion of chemotactic factors, including HETE and possible leukotrienes, or produce complement components. C_{5a} once present can also stimulate macrophages to release chemotactic factor and lysozomal enzymes (39). The net effect

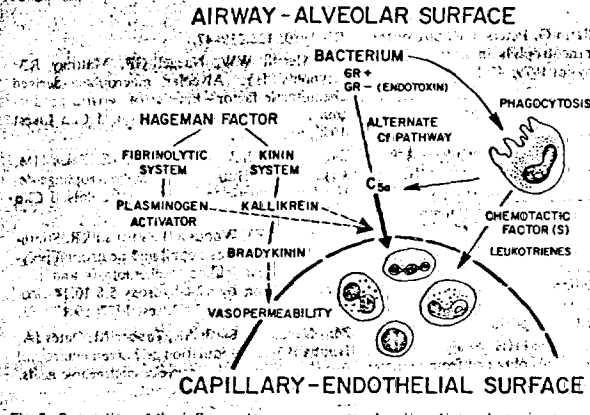

Fig. 5. Generation of the inflammatory response can be viewed as a dynamic process involving both sides of the air-blood interface, but in this illustration the stimulus originates on the airway-alveolar surface and spreads to include cellular and humoral elements in the adjacent capillary and on the endothelial surface. The airway stimulus could be multiple things, but an inhaled or aspired bacterium is an appropriate one (see text for details).

of all these things could be an appreciable increase locally in the alveolus of several factors that possess potent chemo-attractant activity. Either maintenance of a large concentration gradient across the air-blood interface or diffusion of these factors into the vascular compartment could begin the influx of other inflammatory cells into the alveolus. Because the macrophage chemotactic factors studied are rather selective for PMNs, PMN phagocytes seem to be preferentially attracted from the circulating-marginated blood pool. Thus a combination of local macrophage and exogenous PMN phagocytic firepower can be mobilized to the alveolus.

Obviously, many other components of the inflammatory response are present in the alveolus, and mechanisms that could be responsible for them are still poorly understood. Evidence suggests that proteases liberated from PMNs or mast cells can activate a number of Hageman factor-dependent pathways that can initiate the complement sequence, kinin system, and fibrinolysis in addition to the intrinsic pathway of coagulation usually associated with this factor. Whether all of these pathways are operant in the lung is not known conclusively. However, a number of intermediate products formed from these pathways could be relevant in the inflammatory reaction. Plasminogen activator and kallikrein have chemoattractant activity; others have vascular effects that could be important. Products of the kinin system such as bradykinin can alter vascularpermeability that could cause leakage of fluid from the plasma compartment into alveoli. "Inflammation" tends to emphasize cellular components of the reaction, but fluid elements are perhaps of equal importance. Once selective permeability of the endothelial-epithelial surfaces of the blood-air barrier is breached, possibly governed by vasoactive mediators, wholesale transudation of plasma proteins occurs, and high concentrations of many substances important in the inflammatory process, such as complement components, can accumulate.

What becomes crucial is to determine where the fine-tuning of the reaction occurs. What recognizes the specie of bacteria, for example; what causes the switch in phagocytic cell response between macrophages and PMNs; what amplifies the reaction and mobilizes blood cell and plasma resources, and what calls a halt to things once the invasion is contained and gets clean-up and repair under way? In other words, is the reaction a selective and qualitative one with intrinsic control? Alternatively, there may be nothing selective about the inflammatory sequence in the lung, and it is just a quantitative response.

After a certain degree of local injury, the response escalates, albeit in a rather predictable way. Obviously, it is an oversimplification to consider that just one component of lung or systemic host defense is in charge of everything, because biology is more clever than this and several levels of regulation are invariably available. However, the tissue macrophages in the lung are a plausible cellular entity to focus on as potentially a very important point of control in the inflammatory reaction just outlined. What makes this consideration at all feasible stems from the extraordinary versatility of the macrophage itself, which can be at once an effector cell and phagocyte par excellence, and an effector cell capable of secreting a bewildering array of biologic substances (15, 34, 35). Chemotactic factors, complement components, and leukotrienes, selectively considered in this report, constitute only a small number of substances these cells can create.

A small percentage of PMNs is usually found among the respiratory cells retrieved by BAL from lungs of nonsmokers (about 1%) and cigarette smokers (about 3%); since lavage may not completely dislodge all the PMNs, the actual *in situ* number on the alveolar and peripheral airway surfaces is probably greater. But why PMNs should leave their circulating blood pool and migrate into the lung parenchyma and onto the air exchange surface, in the absence of an overt stimulus to attract them, is uncertain. In smokers who inhale burning cigarette gases and particles, the added airway debris probably accounts for the influx of PMNs and the huge increase in the number of macrophages; whereas, in normal nonsmokers small foci of bacteria and occasional particles landing on the alveolar surface could serve as a continuing, low grade stimulus for a very modest inflammatory cell influx. On the other hand, alveolar macrophages are clearly capable of secreting chemotactic factors that seem to preferentially attract PMNs and they could provide one such alveolar-side stimulus. *In vitro*, however, the secretion of chemotactic factors seems nonspecific and can be generated simply by cell adherence and prolonged culture, although certain phagocytic and immune complex stimuli can greatly enhance output.

In vivo, things might be quite different. Macrophages are dispersed throughout the alveoli surface and one cell patrols several alveoli; other macrophages are present in the interstitial and structural areas of the alveoli, presumably undergoing maturation before emerging on the air exchange surface. It is not known if the young, maturing macrophages are as capable as the alveolar ones of secreting chemotactic factors. Whereas, *in vitro*, several million macrophages, activated by having to attach to a foreign surface, seem to produce spontaneously a lot of chemotactically active material in cell culture supernatant, the actual concentration of factors liberated by a single macrophage into its immediate surroundings could be quite minute. Such an amount might not be sufficient to actually attract PMNs into the alveolar space, but the factor could seep into the capillary endothelium and promote sticking of PMNs to that surface. Hence a low level of tonic secretion of chemotactic factors by airside alveolar macrophages might be a mechanism for localizing inflammatory cells such as PMNs in adjacent lung capillaries; more potent stimuli may be required to draw them into lung parenchyma. Within several hours, macrophages can maximally secrete chemotactic factors in response to a phagocytic challenge or IgG containing immune complexes, a situation that might need to occur before PMN influx takes place. In addition, other stimuli including complement fragments, arising independently, may need to act together to promote inflammation. Conceivably, macrophage chemotactic fac-

tors have a dual role: (*1*) to diffuse out of the alveoli surface and aid with margination of PMNs in the vasculature, and (*2*) to accumulate in sufficient concentration intra-alveolarly to create a gradient that will attract cells across the blood-air interface onto the alveolar surface.

The importance of macrophage-derived factors in promoting chronic lung diseases that have persistent low-grade PMN inflammation is likewise unclear. Chronic bronchitis and emphysema associated with cigarette smoking feature elastolytic tissue damage that occurs in part from a combination of excessive leukocytic elastase released from PMNs and inadequate neutralization of it by the protease inhibitor, alpha$_1$ antitrypsin, that is rendered nonfunctional by the smoke (55, 56). Not only do such subjects have a huge increase in the number of alveolar macrophages, but their macrophages are generally more active biochemically (57), and tonic secretion of chemotactic factor seems to be greater. PMN influx into lung tissue might be aggravated. This seems to be a mechanism in patients with idiopathic pulmonary fibrosis who have a high percentage of PMNs in their alveoli and airways, but in this disease an additional stimulus such as an immune complex might be required to promote chemotactic factor secretion.

Nonetheless it seems reasonable to consider ways of blocking the biologic activity of macrophage chemotactic factors. A means of selectively suppressing the macrophage does not appear obvious, although corticosteroids and other immunosuppression may generally reduce chemotactic factor production (33). Alternatively, development of an inhibitor polypeptide or a specific monoclonal antibody might be feasible ways to block this chemotactic activity exogenously. Hopefully, some form of chemotactic inactivator will emerge in the future as a possible therapeutic agent to modulate inflammation.

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